HISTOLOGICAL STUDY ON THE GONOCHORISM OF *SYMPHODUS CINEREUS* (LABRIDAE)

by

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ABSTRACT. - A histological study of the gonadal organization of 163 *Symphodus cinereus* caught in the Bay of Naples between January 1988 and January 1990 demonstrated the absence of sexual inversion in this species. Four phases of gonadal development, related to the seasonal cycle, are described. During the reproductive period the ovaries showed asynchrony in oocyte development, which is typical of serial spawners. The reproductive phase in males begins a month earlier than in females.

RÉSUMÉ. - Une étude histologique de l'organisation des gonades a été effectuée sur 163 *Symphodus cinereus*, échantillonnés dans le Golfe de Naples entre janvier 1988 et janvier 1990. Cette espèce ne change pas de sexe et il y a quatre phases de développement gonadique associées au cycle saisonnier. Pendant la période de reproduction les ovaires montrent un asynchronisme de développement caractéristique des espèces qui pondent plusieurs fois. Le début de la phase de reproduction chez les mâles précède d'un mois celle des femelles.

Key-words. - Labridae, *Symphodus cinereus*, MED, Italy, Reproduction, Gonadal histology, Seasonal cycles.

Symphodus cinereus is a labrid fish commonly found in the Mediterranean where it usually lives on sandy bottoms at the edge of *Posidonia* beds. It is a non-permanent dichromatic species. In winter all *Symphodus cinereus* have the same pale grey-beige colour pattern. During the reproductive period the females and young males are all grey-beige in colour, while the older males have a blue spot on the underside of the tail peduncle and a black dorsal spot below the first spines of the dorsal fin (Lejeune, 1985). The eggs of this species are laid in nests made of algae and are guarded by the male (Fiedler, 1964; Michel *et al.*, 1987).

Quignard (1966) and Remacle (1970) conclude that protogynous sexual inversion may take place in *S. cinereus*, but provide no histological details.

We therefore examined in detail the gonadal organisation of *Symphodus cinereus* in order to study its reproductive cycle. We also looked for signs of the sexual inversion that usually occurs in many species of the labrid family (Atz, 1964; Robertson and Choat, 1974; Reinboth, 1970, 1975).

MATERIAL AND METHODS

A total of 163 specimens of *Symphodus cinereus* were caught in the Bay of Naples at monthly intervals between January 1988 and January 1990. Each individual was measured, weighed, and the colour pattern recorded. The gonads were dissected, weighed and fixed in Smith's solution for 3-5 hours (Mazzi, 1977). After fixation they were

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dehydrated and embedded in paraffin wax. Longitudinal sections, 5-6 μm thick, were stained with haematoxylin and eosin. Staging of the maturation process of germ cells is according to Abu-Hakima (1984). For each individual the gonadosomatic index (GSI) was calculated from the equation: gonad weight x 100/total weight; the mean GSI values are reported for males and females, for each month of the year.

RESULTS

Of the 163 individuals examined, 78 were male (mean length of 7.9 cm) and 85 were female (mean length 7.4 cm).

Gonadosomatic index

In both sexes, the maximum GSI growth rate is between April and May. In males, the GSI reaches maximum values in May, while in females it gradually increases to reach maximum values in June. In males, from May to June, the GSI rapidly starts to decrease. Both sexes reach minimum values in August. In females, the GSI shows a slow, but constant increase from September to April, while in males it starts to increase only in January (Fig. 1).

Histology of the ovary

From February to April the ovary is in the pre-reproductive phase. At the beginning of this period (Fig. 2a) the ovarian lamellae are compact and contain mostly oogonia and oocytes in primary growth phase (Wallace and Selman, 1981). In April many oocytes in early vitellogenesis are also present. During the reproductive period (May-July) all stages of oocyte development are found (Fig. 2b). From April onwards, the ovarian lamellae appear to be enlarged and mostly contain oocytes in late vitellogenesis; post-ovulatory follicular sacs can be seen in May indicating that some females have already begun spawning. In June and July the ovaries are in full reproductive activity and oocytes in all stages of maturation can be observed simultaneously. This type of ovary is called asynchronous (Wallace and Selman, 1981) and spawning may occur several times during the reproductive season (Nagahama, 1983). The presence of atretic bodies, the absence of oocytes in vitellogenesis, and the slightly thickened walls in the ovaries examined, all indicate that the spawning period is over, for most fish, by the end of July. In the post-reproductive period (August-October; see Fig. 2c) the ovary is empty, although some eggs can occasionally be observed. The ovigerous lamellae contain many primary oocytes and oogonia, several of which are in mitosis. In October there is a general thickening of the ovarian wall; the brown bodies and the atretic bodies derived from degenerate primary

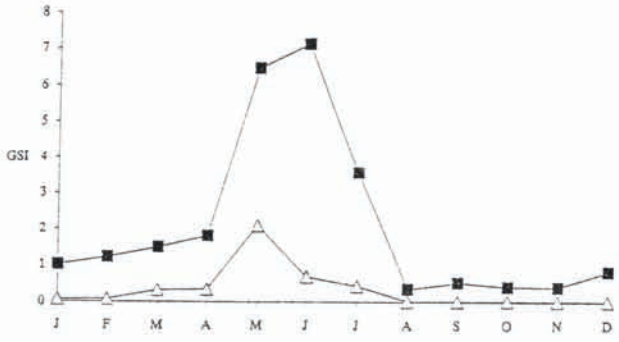

Fig. 1. - Mean monthly GSI for females (■) and males (Δ) of *Symphodus cinereus*.

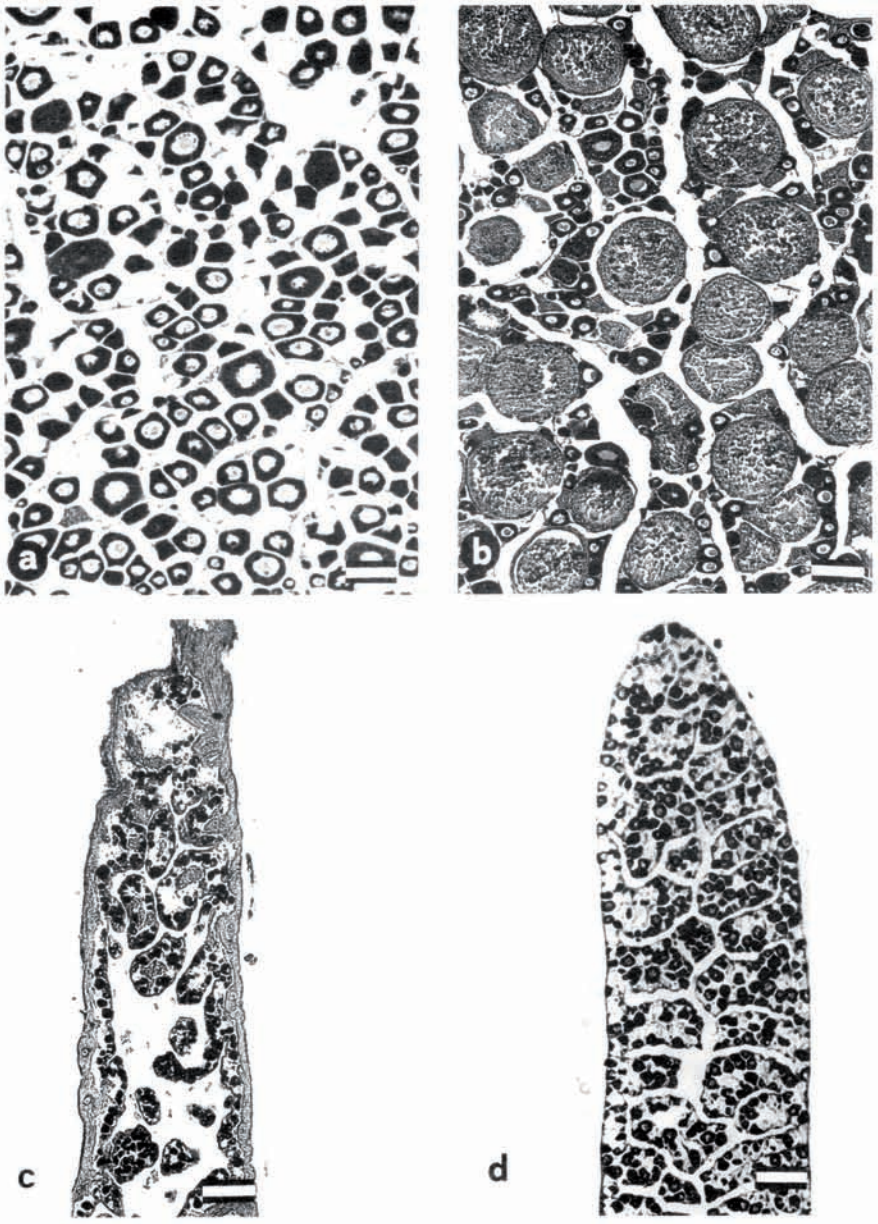

Fig. 2. - Seasonal changes in the structure of the ovary of *Symphodus cinereus*: histological sections; haematoxylin and eosin. a- Pre-reproductive phase (February-April). Ovarian lamellae are compact and contain oogonia and oocytes in primary growth phase; some oocytes are in early vitellogenesis. Scale bar = 91 µm. b- Reproductive phase (May-July). In this period oocytes are present at several stages of maturation, but oocytes in late vitellogenesis are predominant. Scale bar = 205 µm. c- Post-reproductive phase (August-October). The ovary is almost empty; ovigerous lamellae contain mostly primary oocytes. The ovary wall has thickened. Scale bar = 96 µm. d- Quiescent phase (November-January). Ovarian lamellae are filled with resting oocytes. Scale bar = 185 µm.

oocytes can be seen. Between November and January, the ovary is quiescent; the lamellae contain oocytes in resting phase, and atretic bodies are sometimes present (Fig. 2d).

Histology of the testis

The testis of *Symphodus cinereus* is of the "unrestricted spermatogonial tubular type" according to Grier (1981). In the pre-reproductive phase (February-March) the testicle is regular in shape with well-defined tubules predominantly containing spermatogonia. Inside the basal membrane, cysts of dividing spermatogonia are encircled by Sertoli cells. By the end of March most tubules have cysts of primary and secondary spermatocytes (Fig. 3a). During the reproductive period (April-July) the tubules enlarge and the cysts mature. In April and May many spermatogonia and spermatocytes are present but the tubules predominantly contain spermatozoa. All the different stages of maturation can be seen during June and July; there are fewer spermatocytes and the lumen is filled with mature sperm. By the end of July, only a small quantity of sperm remains in the tubules. During this final phase the interstitial tissue thickens (Fig. 3b). At present we do not know which cells are responsible for interstitial thickening, because we have not conducted histochemical studies on these preparations. At the beginning of the post-reproductive period (August-October), the tubules contain only mature sperm (Fig. 3c); towards the end of this period, the testicle seems to be a mass of spermatogonia empty of sperm. During quiescence (November-January) the tubules, containing only spermatogonia, become smaller and growth starts again in January (Fig. 3d).

No gonads showed signs of sexual inversion. The testis of 78 males showed primary structure without any evidence of prior oogenesis, according to the histological parameters of Reinboth (1967) and Dipper and Pullin (1979). None of the 85 ovaries examined showed any signs of regression and none of them was at the onset of testicular development.

DISCUSSION

Histological analysis of *Symphodus cinereus* gonads revealed four phases of the seasonal cycle: pre-reproductive, reproductive, post-reproductive and quiescent, as reported for *S. ocellatus* (Bentivegna and Benedetto, 1989). Except for the reproductive period, the annual growth cycle of the GSI was similar in the two species. In fact, male and female *S. ocellatus* enter the reproductive period together, thus presenting peak GSI values at the same time (Bentivegna and Benedetto, 1989), while *S. cinereus* males enter their reproductive phase earlier than females and gonad maturation and maximum values are reached approximately one month earlier than in females. This is probably because, before females are ready to reproduce, males must be sufficiently motivated to delimit their nesting territory which is larger than that of other species of *Symphodus* (Michel, 1981).

Histological studies conducted in *S. cinereus* showed that in the pre-reproductive period, the onset of maturation of the germinal stages occurs simultaneously in the testicle and in the ovary. Females spawn several times during the reproductive season as shown by asynchrony in development of the oocytes (Nagahama, 1983). The existence of more than one cycle during the reproductive period has been confirmed by behavioural observations (Michel and Voss, 1982).

Taken together, the distribution of sexes in relation to body size, the lack of signs of gonad inversion and the primary structure of testicles, indicate that *Symphodus cinereus* does not undergo sexual inversion.

The gonochorism observed in species of *Symphodus* is different from that found in other Perciform families, as, for instance, Serranidae and Sparidae. In fact, in Sparidae remnants of the bisexual juvenile phase persist into the adult stage (D'Ancona, 1949a;

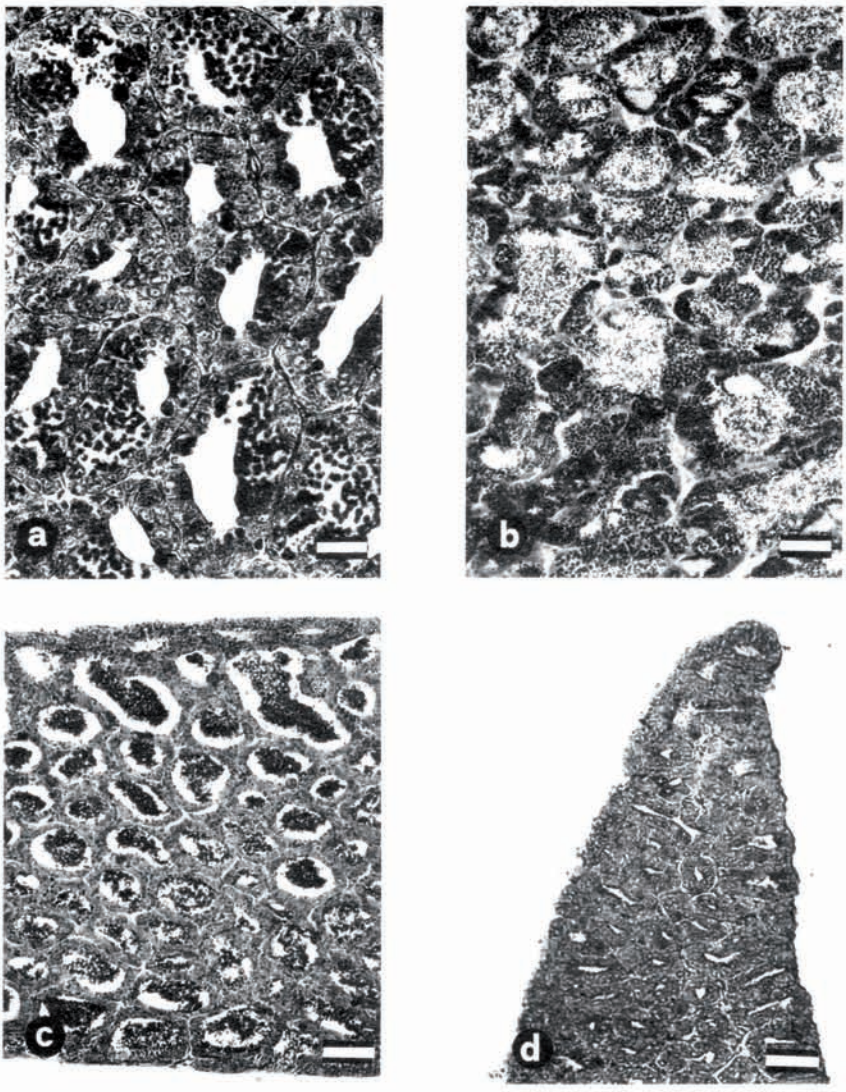

Fig. 3. - Seasonal changes in the structure of the testis of *Symphodus cinereus*: histological sections; haematoxylin and eosin. a- Pre-reproductive phase (February-March). Seminiferous tubules are enlarged and regular and show cysts of spermatogonia or of primary spermatocytes. Scale bar = 28 µm. b- Reproductive phase (April-July). Tubules contain all stages of sperm maturation; the lumen is filled with mature sperm. Scale bar = 49 µm. c- Post-reproductive phase (August-October). In this photo, the testis shown is just entering the post-reproductive phase; the tubules contain only sperm remains. Scale bar = 40 µm. d- Quiescent phase (November-January). Tubules are restricted, almost closed, and contain only spermatogonia. Scale bar = 45 µm.

Reinboth, 1962; Atz, 1964) and in Serranidae gonochorism seems to derive from hermaphroditism (Smith, 1971; Smith and Young, 1966).

The lack of sex inversion in some species of *Symphodus* can be related to the highly specialised reproductive system required for reproductive success (Chan and Yeung, 1983). In *S. cinereus* the male must defend a vast territory, build an elaborate nest, attract females, and guard the eggs. According to Warner and Lejeune (1985),

extensive male mating-investment limits the extremes of male reproductive success, and thus reduces selection for protogynous sex change.

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